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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 71007/137/USGO

In re patent application of

Apurba BHATTACHARJEE *et al.* Group Art Unit: 1641

Serial No.: 08/886,044 Examiner: S. Devi

Filed: June 20, 1997

For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

I, **ALAN S. CROSS, M.D.**, of 6810 Brookville Road, Chevy Chase, Maryland 20815, do solemnly and sincerely declare that:

1. I am one of the inventors of the inventions disclosed and claimed in the patent application captioned above. My *Curriculum Vitae* is attached to a prior declaration.

2. Attached as Exhibit 1 are Methods and Results relating to active immunization with a detoxified *Escherichia coli* J5 LPS-Group B meningococcal outer membrane protein complex vaccine, and subsequent challenge with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

3. Active immunization with J5 dLPS/OMP vaccine produced a prompt and sustained anti-core glycolipid antibody level that was generally in 100-fold excess of pre-immunization baseline levels. Twenty-four hours after onset of bacteremia, antibody levels decreased, but then rapidly recovered to, and remained at, pre-infection levels. Active immunization with J5 LPS/OMP vaccine induced greater than 800 ELISA units/ml of antibody at the onset of neutropenia, nearly 4 weeks after the last dose of vaccine, and this

level persisted throughout the entire period of neutropenia, for up to 80 days after the initial immunization. This is in distinct contrast to results achieved by passive immunization with antibodies, where initial levels of 800 ELISA units/ml of antibody dropped to less than 200 ELISA units/ml of antibody by 24 hours.

4. Immunization did not prevent either systemic infection or initiation of sepsis, but it did reduce the likelihood of a lethal outcome following infections with both heterologous strains of bacteria. Vaccinated animals challenged with *Pseudomonas aeruginosa* had an overall survival rate of 48% compared to 7% for saline treated control animals. A similar result ensued with *Klebsiella pneumoniae* challenge, with a 64% survival rate for vaccinated animals versus a 13% survival rate for control animals.

5. Vaccinated animals had the same frequency and magnitude of bacteremia from the challenge strain as the control group, but had significantly lower levels of bacteria in liver and spleen than control animals. I hypothesize that antibodies generated in response to the vaccine promote the uptake and killing of bacteria from the blood by tissue.

6. In addition to the decreased bacterial levels in liver and spleen, there was a significantly lower level of circulating endotoxin at the onset of fever in vaccinated animals as compared to control animals. While endotoxin levels increased in both groups at 24 hours, they were still lower than those of the control group. The lower level of circulating endotoxin may be due in part to promotion of LPS clearance from the circulation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

1/12/99
Date

Alan S. Cross, M.D.